

# Anxiolytic Potential of Methanol Extract from *Ageratum conyzoides* Linn Leaves

Ravinder Kaur<sup>1</sup> and Sarabjit Kaur<sup>1\*</sup>

Department of Pharmaceutical Sciences, Pharmacognosy and Phytochemistry Laboratory, Guru Nanak Dev University, Amritsar-143005, INDIA.

## ABSTRACT

**Objective:** *Ageratum conyzoides* Linn. (Asteraceae) has been widely used in African traditional medicine for healing mental and infectious diseases. The present study was designed to scientifically validate the traditional claim of *A. conyzoides* as anti-anxiety drug and to identify the compound responsible for the anxiolytic effects of *A. conyzoides*. **Method:** The methanol extract of *A. conyzoides* was prepared by soxhlet apparatus. The methanol extract was fractionated into ethylacetate and butanol fractions by liquid-liquid partitioning method. Methanol extract (100 and 200 mg/kg; p.o.) and its prepared fractions (25 and 50 mg/kg; p.o.) were evaluated for anxiolytic activity in mice by using elevated plus maze (EPM) model. Thin layer chromatography studies were performed to identify the possible anxiolytic component. **Results:** Methanol extract at both doses showed significant, when compared to vehicle control group, increase in time spent and number of entries in open arms of EPM confirming the anti-anxiety effects of *A. conyzoides*. Liquid-liquid partitioning of methanol extract gave two fractions (ethylacetate and butanol) which were administered at 25 and 50 mg/kg doses to mice in EPM, respectively. Results showed that ethylacetate fraction was responsible for anxiolytic effects of methanol extract of *A. conyzoides*. The TLC studies were carried out for ethylacetate fraction and Quercetin was identified by comparing  $R_f$  values with the standard (Quercetin). **Conclusion:** The present investigation revealed that the extract has significant anxiolytic effect. The flavonoid quercetin may be responsible for the observed anxiolytic effects of *A. conyzoides*.

**Key words:** *Ageratum conyzoides*, Anxiolytic, Methanol extract, Quercetin, TLC.

## INTRODUCTION

Anxiety disorders, nowadays, are one of the common causes for frailty. Nearly one fourth of the adult population suffers from these psychiatric disorders during the course of their life. Women are more prone to anxiety disorders (30.5%) as compared to men (19.2%). Research data revealed that very few people (<14%) suffering from such disorders receive treatment.<sup>1</sup> Due to worldwide increase in the incidence of anxiety disorders, reaching about 16.6%, advancements have been made regarding their causes and treatments.<sup>2</sup> Cognition, psychomotor function and intellect, all are hampered by anxiety. Benzodiazepines are

considered top notch among all the existing drug treatments for anxiety.<sup>3</sup> But there are numerous side effects linked with the present treatment of anxiety such as cardiotoxicity, hypertension, blood dyscrasia, impairment of memory and attention.<sup>4</sup> The past era has encouraged the practice of complementary and alternative medicine. The ability of herbal drugs in recuperating various behavioral disorders has been bolstered by plentiful of research investigations.<sup>5-7</sup>

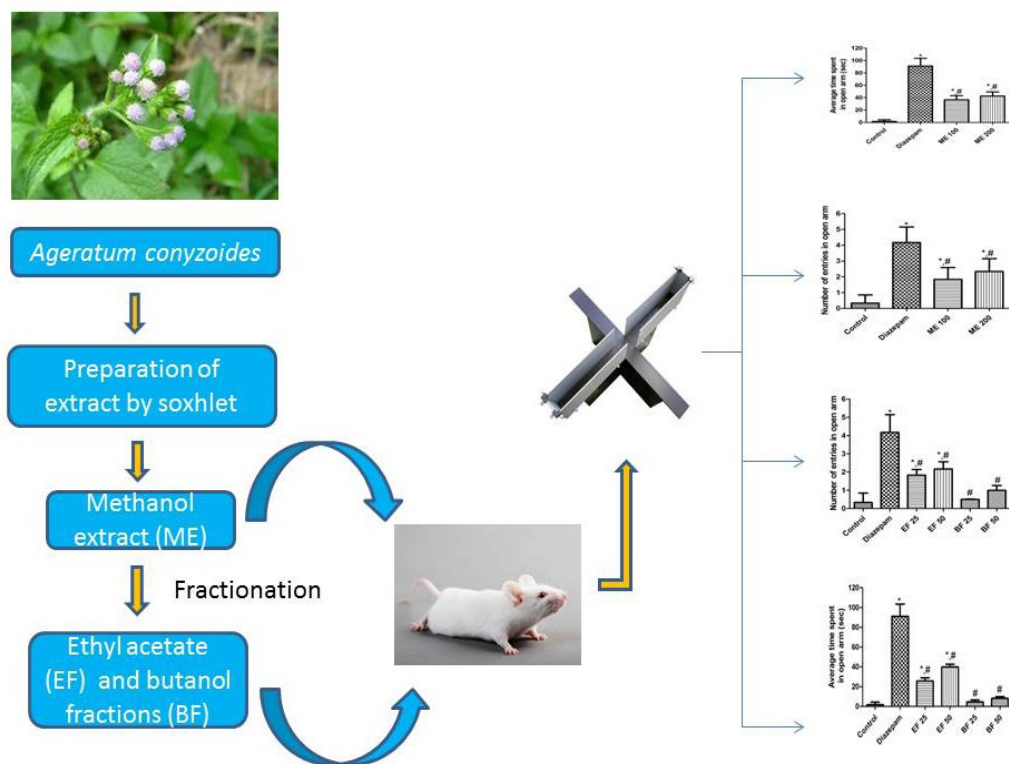
*Ageratum conyzoides* is an annual branching herb belonging to family Asteraceae with nearly 90 cm height.<sup>8</sup> The plant is commonly known as Goatweed, Floss flower or Whiteweed.<sup>9</sup> It has been used customarily in certain African realms for the treatment of psychological and contagious ailments.<sup>10</sup> Aerial parts of the plant are used to cure diabetes.<sup>11</sup> Plant foliage is used to heal cuts and injuries as well as an antidote for snake bite.<sup>12</sup> Leaves are also used for blood coagulation.<sup>13</sup> Reports reveal that the

### \*Corresponding author:

Mrs. Sarabjit Kaur

Assistant Professor, Department of Pharmaceutical Sciences,  
Pharmacognosy and Phytochemistry Laboratory,  
Guru Nanak Dev University, Amritsar-143005, INDIA.  
E-mail: gndu\_sarabjit@yahoo.co.in

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Graphical Abstract

leaves of the plant act as mines full of flavonoids namely quercetin, quercetin-3-rhamnopyranoside, kaempferol, kaempferol-3-rhamnopyranoside, 5'-methoxynobiletin, linderoflavone B.<sup>10</sup> There are traditional claims for the root of the plant being used as anthelmintic and antidysenteric.<sup>14</sup> Recently, Varadharajan and Rajalingam, (2011) reported the antiepileptic potential of *A. conyzoides* in rats.<sup>15</sup>

Hence, the present study has been designed to scientifically validate the traditional claim of *A. conyzoides* as antianxiety agent.

## MATERIALS AND METHODS

### Plant material

Leaves of *Ageratum conyzoides* were collected from local areas around the Guru Nanak Dev University, Amritsar, Punjab, India. The taxonomic identity of plant was confirmed by Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab, India. A voucher specimen no S.R. Bot Sci/97 herb has been deposited in the herbarium of Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab, India. Leaves were dried under shade and milled in grinder to fine powder.

### Preparation of Extracts

The powdered leaves (500 g) were subjected to successive Soxhlet extraction for 48 hours by using solvents in increasing polarity viz. petroleum ether (60-80°C) and methanol. Each extract was concentrated by distilling off the solvent using rotavapour and then evaporated to dryness on the water-bath. Extracts were weighed and percentage yield was calculated in terms of the air-dried weight of the plant material.

### Fractionation of the methanol extract

Twenty two grams of the methanol extract was fractionated by dissolving in 100 mL of distilled water. Then extraction was done with hexane (600 ml) and aqueous phase was collected. After that successive liquid extraction of aqueous phase was done with ethyl acetate (3 X 150 ml) and butanol (3 X 150 ml). Ethyl acetate fraction of yellow color and butanol fraction of red color were concentrated using a rotary evaporator.

### Phytochemical screening

All extracts and fractions were subjected to phytochemical screening to detect the presence of various phytochemicals viz. alkaloids, flavonoids, tannins, anthocyanins and saponins.<sup>16,17</sup>

### Drugs

Diazepam (DZP, 2.0 mg/kg, Sigma, India) was used as the standard anxiolytic drug (positive control group). Carboxymethylcellulose (CMC, 1% w/v, Merck, India) was employed to treat negative control group. All other solvents and reagents were of analytical grade.

### Animals and treatments

Swiss Albino mice weighing 25-30 g were procured from Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. Eight animals per cage were held and maintained under laboratory conditions at 25°C with a normal 12 h light/dark schedule and free access to water and food. Mice were allowed to acclimatize to the laboratory environment (for at least 3 weeks) prior to the commencement of experimentation. Six mice per group were used in all sets of experiments. Experiments were carried out in a noise free area with controlled lighting, between 8:00 a.m. and 12:00 p.m. All protocols and experiments were conducted in strict compliance according to the ethical principles and guidelines provided by Institutional Animal Ethical Committee (IAEC). The conditions required to obtain reliable data i.e. least number of animals and time of surveillance, were utilized.

The anxiolytic activity of methanol extract and its fractions (ethylacetate and butanol) were evaluated by employing EPM. All the test and standard drugs were administered to mice 30 min prior to experiment via oral and intraperitoneal route respectively. Various animal groups employed in the present study were as follows:

Group I—Vehicle treated: CMC (1 % w/v) was administered orally

Group II and III—Methanol extract (100 and 200 mg/kg)

Group IV and V—Ethylacetate fraction of methanol extract (25 and 50 mg/kg)

Group VI and VII—butanol fraction of methanol extract (25 and 50 mg/kg)

Group VIII—Positive control: diazepam (2 mg/kg)

### Elevated plus-maze (EPM)

Selection of drugs that help in modifying anxiety is done with the help of EPM, the most extensively employed model in understanding the psychological and neurochemical basis of anxiety.<sup>18</sup> The EPM model used in the study consisted

of two open (30 x 5 x 0.2 cm) and two closed (30 x 5 x 15 cm) arms, stretching out from a platform (5 x 5 cm) in the midway and raised to a height of 45 cm above the ground.<sup>19</sup>

Each animal was positioned at the center of the maze facing one of the enclosed arms, thirty minutes after administration of drugs. Number of entries and time spent in closed and open arms were recorded for 10 min. Entry into an arm was stated as the animal placing all four paws on the arm. The maze was cleaned properly with 5% alcohol, each time before placing the animal, to abolish the possible prejudice due to the scent left by the preceding animal.

### Statistical analysis

All data are expressed as mean  $\pm$  standard deviation (SD) and analyzed statistically by one way analysis of variance (ANOVA) followed by Tukey's Multiple Range test using Graph Pad Prism 5.0 software. A p value of <0.05 was considered statistically significant.

## RESULTS

### Percentage yield and phytochemical screening of prepared extracts and fractions

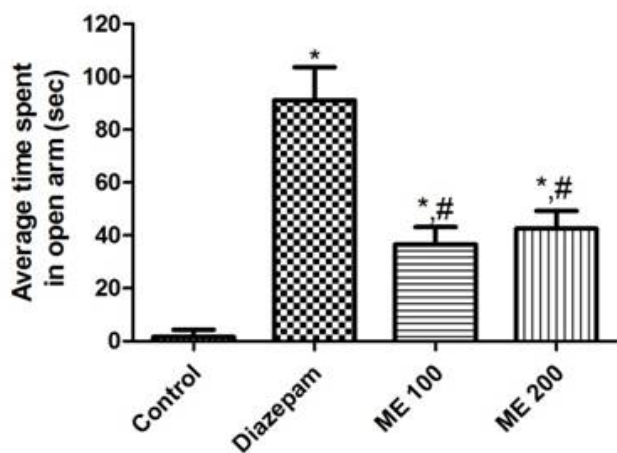
The methanol extract gave the yield of 10% w/w and its ethylacetate and butanol fractions gave 0.6% w/w and 0.4 % w/w, respectively. Preliminary phytochemical screening of methanol extract revealed the presence of numerous phytochemical groups (Table 1). Both the fractions of methanol extract showed the presence of flavonoids.

### Evaluation of anxiolytic activity of methanol extract and its fractions

The methanol extract of *A. conyzoides* showed significant increase in average time spent and mean of number of entries by mice in open arms when compared to control group (Figure 1 and 2) confirming the traditional claim of plant. However, the observed anxiolytic effect at dose of 100 mg/kg of methanol extract was not significantly different when compared to anxiolytic effect of same extract at dose of 200 mg/kg. Administration of diazepam (2 mg/kg) to mice significantly ( $p < 0.05$ ) increased the mean time spent by mice in open arms and the average number of entries into the open arms of EPM, confirming an anxiolytic effect.

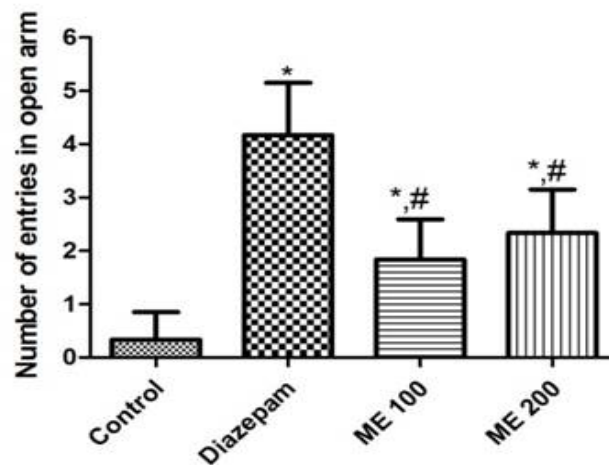
Among the prepared fractions of methanol extract, only ethylacetate fraction (25 and 50 mg/kg) showed significant ( $p < 0.05$ ) anti-anxiety activity which was confirmed by increase in average time spent and mean of number of

Phytoconstituent	Methanol extract	Ethyl acetate fraction	Butanol fraction
Alkaloids	+	-	-
Carbohydrates	+	-	-
Protein and amino acids	+	-	-
Phytosterols	+	-	-
Flavonoids	+	+	+
Terpenoids	+	-	-
Tannins	+	+	+



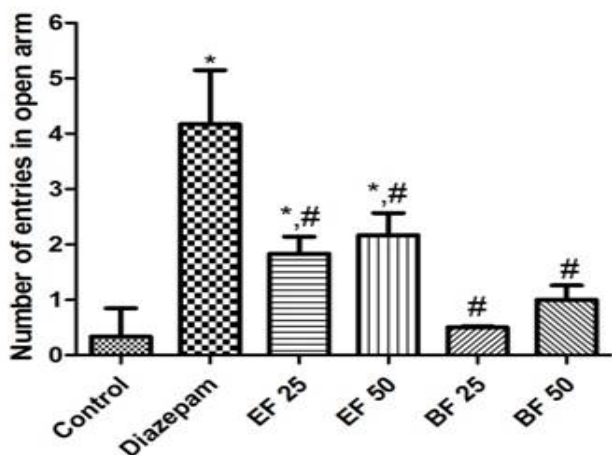
**Figure 1: Effect of methanol extract of *Ageratum conyzoides* on average time spent in the open arms by mice exposed to the EPM test**

Data are expressed as mean ± S.D; n=6; Anova followed by Tukey's *post hoc* multiple range test; \*p<0.05 versus control; #p<0.05 versus Diazepam; ME 100=methanol extract 100 mg/kg; ME 200=methanol extract 200 mg/kg.



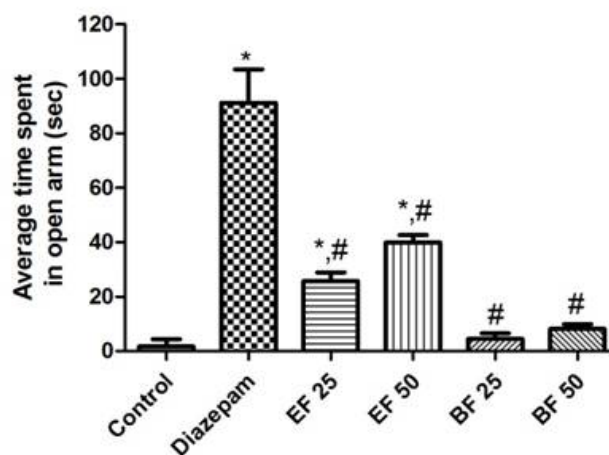
**Figure 2: Effect of methanol extract of *Ageratum conyzoides* on number of entries in open arms by mice exposed to the EPM test**

Data are expressed as mean ± S.D; n=6; Anova followed by Tukey's *post hoc* multiple range test; \*p<0.05 versus control; #p<0.05 versus Diazepam; ME 100=methanol extract 100 mg/kg; ME 200=methanol extract 200 mg/kg.



**Figure 3: Effect of ethyl acetate and butanol fractions of methanol extract of *Ageratum conyzoides* on average time spent in the open arms by mice exposed to the EPM test**

Data are expressed as mean ± S.D; n=6; Anova followed by Tukey's *post hoc* multiple range test; \*p<0.05 versus control; #p<0.05 versus Diazepam; EF 25 = ethyl acetate fraction 25 mg/kg; EF 50=ethyl acetate fraction 50 mg/kg; BF 25=butanol fraction 25 mg/kg; BF=butanol fraction 50 mg/kg.



**Figure 4: Effect of ethyl acetate and butanol fractions of methanol extract of *Ageratum conyzoides* on number of entries in open arms by mice exposed to the EPM test**

Data are expressed as mean ± S.D; n=6; Anova followed by Tukey's *post hoc* multiple range test; \*p < 0.05 versus control; #p<0.05 versus Diazepam; EF 25=ethyl acetate fraction 25 mg/kg; EF 50=ethyl acetate fraction 50 mg/kg; BF 25=butanol fraction 25 mg/kg; BF=butanol fraction 50 mg/kg.





**Figure 5: Comparison of TLC profile of Quercetin (left side) and ethylacetate fraction (right side) of methanol extract**

entries by mice in open arms of EPM when compared to control group. The butanol fraction of methanol extract of *A. conyzoides* did not show any significant anti-anxiety activity (Figure 3 and 4).

#### *Thin layer chromatography study of ethylacetate fraction of methanol extract*

TLC chromatogram of ethylacetate fraction of methanol extract and standard flavonoid i.e. quercetin was developed by using toluene: ethylacetate: formic acid in ratio of 10:3:1 v/v/v as mobile phase. The developed chromatogram of ethylacetate fraction of methanol extract showed the presence of quercetin. This was confirmed by comparing  $R_f$  values of ethylacetate fraction with standard quercetin (Figure 5).

## DISCUSSION

*Ageratum conyzoides* is a plant species that is broadly employed as a remedy for mental disorders. In the present study, *Ageratum conyzoides* was evaluated for anxiolytic effect in order to scientifically validate the traditional claim by using behavioral models namely elevated plus maze. The oral administration of methanol extract (100 mg/kg and 200 mg/kg) to mice showed anti-anxiety effects indicated by increase in average time spent and number of entries in open arm of EPM. Phytochemical screening of methanol extract showed the presence of flavonoids along with other phytochemical groups. The chemical separation of the active extract allowed isolation of an active fraction (ethylacetate fraction) that exhibited significant anxiolytic properties. TLC evidenced that flavonoids comprise the major group of compounds present in the active fraction. By comparing the  $R_f$  value of standard Quercetin with that of ethylacetate fraction, it was confirmed that quercetin was found to be the major compound present in ethylacetate

fraction of methanol extract.

Various *in vivo* studies have recognized flavonoids as novel type of ligand with anti-anxiety outcomes. Behavioral tests in rodents have explored anxiolytic effects of different flavones (e.g., chrysin and apigenin) obtained from medicinal plants. The biological effect produced by these compounds is due to the modulation of GABA ( $\gamma$ -amino butyric acid) ergic system.<sup>20</sup> Neuroprotective manifestation of flavonoids has been attributed to their general bioavailability and *in vivo* occurrence in the brain.<sup>21</sup> Quercetin, one of the flavonol found in large number of herbals, act as a monoamineoxidase A and B (MAO A and B) inhibitor.<sup>22</sup> Research has revealed that substances acting as MAO modulators elicit behavioral alterations in rodents by modifying monoamine level in brain, and consequently display an anxiolytic effect.<sup>20</sup> Recent findings have proposed that MAO-inhibition and enhancement in GABA ergic activity may be the fundamental mechanism responsible for anxiolytic activity of quercetin liposomes administered via nasal route.<sup>23</sup>

Result of the present study indicated that the methanolic leaf extract of *A. conyzoides* had central anxiolytic effects. The phytoconstituent that may be responsible for the observed anti-anxiety effect has been detected in ethylacetate fraction of methanol extract and identified as a well-known compound, Quercetin, a flavonol.

## CONCLUSION

In conclusion, the present study validates the traditional claim of *Ageratum conyzoides* as anxiolytic drug as methanol extract of leaf produced a significant anti-anxiety effect. Chemical fractionation identified quercetin as one of the major compound that may be responsible for the observed anti-anxiety effect of plant. Since there is a necessity for new safer and cost effective anxiolytic compounds having few side effects as compared to synthetic medication, *A. conyzoides* can act as a potential lead for drug development.

## CONFLICT OF INTEREST

The authors have no conflict of interest.

## ACKNOWLEDGEMENT

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## Highlights of Paper

- *Ageratum conyzoides* Linn., commonly known as Jangli Pudina, is an important ethnomedicinal plant belonging to sunflower family i.e. Asteraceae.
- The leaves of the plant were found to be anxiolytic at 200 mg/kg p.o. dose.
- Anti-anxiety effect of the extract may be due to the presence of flavonoids in the fraction (obtained from extract) as confirmed by TLC.

## Author Profile



- **Mrs. Sarabjit Kaur:** Presently working as Assistant Professor in the Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, Punjab, India. She has six publications to her credit of National and International repute. She has also presented six papers in national and international conferences.



- **Ravinder Kaur:** Currently working as a Research fellow in UGC BSR Fellowship Scheme 2013-14 granted to Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab, India. Also pursuing PhD from the same department.

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